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THEORETICAL STUDIES OF MOLECULAR MECHANISMS OF DNA DAMAGE INDUCED BY HYDROXYL RADICALS

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Hydroxyl radicals can induce damage in DNA by two primary reactions. One is through hydrogen abstraction from the deoxyribose, which leads to the formation of a carbon-centered radical, the other is through addition to the pyrimidine bases of DNA. Both processes lead to DNA damage; but, while hydrogen abstraction leads almost directly to a strand break, the addition of hydroxyl radicals to a pyrimidine base (e.g., thymine) may induce a strand break through a complicated sequence of events.

In order to elucidate the molecular determinants for the reactivity of the various components of DNA, that interact with a hydroxyl radical, and to identify the intermediates produced from such interactions, we study these primary processs with quantum chemical methods.

Hydrogen abstraction

The reactive site of a deoxyribose, which is susceptible to hydrogen abstraction, is the H_4'. Hydrogen abstraction at this site leaves a radical centered on C_4', which may lead to a strand break through a beta-cleavage process.[1] The hydrogen abstraction is modeled on a truncated deoxyribose in which only the neighbors of C_4' are kept.

Simulations of the approach of a hydroxyl radical along the C_4'H_4' bond shows that the interaction is repulsive in the entire region that was investigated. The repulsive energy of the system increases monotonically as the distance between OH and C_4' decreases. The barrier to the hydrogen abstraction, on the other hand, investigated at three different C_4'-O distances is found to become smaller as the two reacting molecules approach each other. At the shortest distance investigated (C_4'-O = 2.5 Å) the barrier disappears completely and hydrogen abstraction becomes spontaneous. However, the repulsion at this distance is 10.3 kcal/mole. On this potential energy surface, describing the approach of the hydroxyl radical and the abstraction of the hydrogen, we identified the transition state. This point on the potential surface represents an optimal balance between the cost to overcome the repulsive force and the cost to overcome the barrier to hydrogen transfer.

Hydroxyl addition to bases

Hydroxyl radicals add preferentially to the $C_5 = C_6$ bond of pyrimidine bases. This addition is very rapid and is probably the primary reaction in the damage to nucleic acid bases induced by hydroxyl radicals. We have selected to investigate the addition of OH to uracil. The addition at either the C_5 or the C_6 end of the double bond produces radicals with different properties and at markedly different yields. The radical produced by addition at C_5 is reducing and is obtained at a yield of 80%. The radical produced by addition at C_6 is oxidizing and its yield is only 20%.[2]

The simple explanation for the selectivity in sites of addition is based on the electrophilic properties of OH and the higher net charge on C_5 than on C_6; it does not seem to hold when subjected to rigorous scrunity by our calculations. In particular, because the addition of OH to a double bond proceeds through its interaction with the pi electrons, the larger pi charge on C_6 than on C_5 does not support the explanation based on charge density.

We have therefore simulated the process of addition of OH to the C_5 and C_6 positions of uracil. The structures of the corresponding products and the transition states of the addition reaction were optimized. The energies of both the product and the transition state of the 5-hydroxy-6-uracilyl radical are lower than the energies of the corresponding states of the 6-hydroxy-5-uracilyl radical. This result is consistent with the experimental findings. However, the energetic difference is small and strongly dependent on the contributions from electronic correlation and probably on the basis set selected for the computations.

The analysis of the structural changes in the transition states and the products of the OH addition reaction provides a mechanistic explanation for the preference of the addition to C_5 over C_6. The transition state of the addition occurs when the OH approaches the planar ring to approximately 1.85 A. At this distance the interaction between OH and the uracil causes a distortion of the ring. The products of the addition, i.e., the uracilyl radicals, are distorted in a way consistent with the formation of a new tetrahedral carbon. However, while the distortions in the transition state and in the product of the 5-hydroxy-6-uracilyl radical are of a similar nature, the transition state of the 6-hydroxy-5-uracilyl is distorted in an opposite direction from that in the product of this reaction. The results suggest that the addition of OH to C_5 proceeds smoothly through the transition state to the products, whereas the addition to C_6 proceeds to a transition state which needs to invert the distortion of the ring before it can complete the reaction to yield the products. Thus, the final formation of the C_6–OH bond may be strongly coupled to ring inversion and the reaction may need to pass through another transition state. The mechanistic description of the differences in the addition of OH to C_5 and C_6 may explain the observed preference of this reaction.

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